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No. I.—Contributions to the Morphology and Physiology of the Cell.
By A. B. MACALLUM, M.B., Ph.D.

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[*Extract from Transactions of the Canadian Institute, 1890.*]

CONTRIBUTIONS TO THE MORPHOLOGY AND PHYSIOLOGY OF THE CELL.

BY A. B. MACALLUM, B.A., M.B., Ph.D.,

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(Read 15th November, 1890.)

In the interior of the epithelial cells of the alimentary canal, and in the glandular cells of the pancreas in amphibia, are usually found structures which are of great interest to both the morphologist and the physiologist. Typical examples of these occurring in the gastric mucosa of the salamander have been described and illustrated by Lukjanow,* and one has but to glance over the figures he has given in order to gain an idea of the number and variety of these bodies. They are much more abundant in the intestinal than in the gastric epithelium of a well-nourished animal, and, so far as my observations go, they present, on the whole, a greater complexity of form than those described by Lukjanow. What is the significance of these bodies? With the exception of some of the intranuclear forms, they can, I believe, be arranged in the three following divisions:

1. Parasites.
2. The remains of broken-down cells and nuclei swallowed by the healthy adjoining cells.
3. Material swallowed by the epithelial cell from the food passing over its free surface (in the case of the intestinal epithelium).
4. Plasmosomata migrated or extruded from the nucleus (only in the glandular cells of the pancreas).

It is, no doubt, impossible, in many cases, to determine to which of these classes this or that particular body belongs since intracellular parasites simulate plasmosomata and kindred structures in some stages of their existence, and I propose, therefore, to treat of the structures in a general way, pointing out, wherever possible, their relationship to one or other of the classes given above excepting, however, those connected

* Beiträge zur Morphologie der Zelle—Arch. fur Anat. und Phys., Suppl. Bd. zur Phys. Abth., 1887, p. 66.

with division 3, the treatment of which I postpone until I have finished my experiments on the methods of the resorption of chromatins *(Nucleins)*.

To illustrate the parasitic nature of some of these forms, I will now describe undoubted examples of intracellular parasites from the intestines of the spotted newt and the lake lizard *(Necturus)*.

I. A CELLULAR PARASITE FROM THE INTESTINAL EPITHELIUM OF DIEMYCTYLUS VIRIDESCENS.

In April of this year I obtained from the neighborhood of Toronto a number of spotted newts for the purpose of studying the phenomena of secretion in the pancreas and in making preparations of this organ I found it frequently convenient, on account of the small size of the animal and its organs, to include the anterior portion of the intestine. In the intestinal epithelium of one of the newts was found a large number of forms like those shown in Figures 3 and 4, and I immediately endeavored to work out their history. Before detailing the results of this work it may be well to state that the particular object from which the sections studied were made was hardened in Flemming's Fluid and alcohol, stained *in toto* with hæmatoxylin, imbedded by the chloroform process in paraffin, the sections therefrom placed in series on the slide and stained with eosin and safranin, before being permanently mounted in balsam.

The structures in question are so numerous that every second or third epithelial cell, for long stretches of the section, contained one of them. They are always placed in the outer half of the cell between the nucleus and the free border, and have a nearly uniform diameter (9-11μ, averaging 10μ) and an approximately spherical shape. They do not appear to have a definite or distinct membrane, and what takes its place appears to be a zone of homogeneous or faintly granular protoplasm which, in many cases, is denser and thicker at one side of the body than at any other. From this zone trabeculae of granular protoplasm pass inwards to terminate in a more or less centrally placed protoplasmic mass. In a number of these bodies sufficient to render the peculiarity prominent, the bulk of the protoplasm is collected at one side (Fig. 3), while the thicker portion of the protoplasmic rim occupies the opposite side with a large crescentic, oval, or round cavity intervening. The protoplasmic mass stains lightly but readily with eosin and contains a round homogeneous nuclear body, which stains deeply with safranin and measures less than 2μ (1.5μ). Sometimes the nuclear body is placed in a cavity in the protoplasmic mass and connected with the latter by a few fine strands. In a few instances, the nucleus was surrounded at a distance by

a distinctly marked membrane which, however, may have been only a thickening of the protoplasm bordering the cavity in which the nucleus was situated.

Not so common, but still quite readily seen, are forms like that represented in Fig. 5, in which, in place of a single nucleus, there are a large number (over twenty) of safranophilous spherules, each surrounded by a small quantity of finely granular protoplasm and marked off from the rest of the mass by a delicate membrane (Fig. 8*d*.) These spherules are homogeneous and measure much less than 1μ. Fig. 6 apparently represents a later stage of the same body, and in this one sees that the homogeneous spherules have become transformed in such a way, that the stained material in each is arranged in a horseshoe or crescentic form, according to the specimen examined (Fig. 6, 7, 8*e*). The comparative scarcity of these forms, the very small size of the objects and the absence of a sharply defined contour to the stained material, render it extremely difficult to determine this arrangement satisfactorily in many cases, but in thin and well stained sections, and with good objectives (2mm. immersion apochromatic, Zeiss), forms like those figured appear now and then.

There can, I think, be no doubt about the parasitic nature of these intracellular bodies, and we may, therefore, regard the stage described in the last paragraph as that of sporulation.

I endeavored to determine the mode of transition from the stage in which there is a single nucleus to that of sporulation. It was not an easy subject for study, because, for every hundred that one observes belonging to both stages, there are not more than one or two forms that can be ranked as transitional. Two of such are represented in Fig. 8*b* and *c*. I have been led to consider them as stages in the formation of spores, because they present structures which resemble somewhat karyokinetic figures. For example, in the form represented in Fig. 8*b*, the centrally placed stained body may be regarded as belonging to the dyaster stage and seen from one of the poles; in it also structures, bearing a resemblance to individual chromatin loops, can be made out. This arrangement comes out well sometimes in preparations stained with hæmatoxylin and safranin, but oftener the safranophilous substance is collected in a ring form resembling, to a certain extent, the equatorial plate of nuclear division. Probably the explanation of Fig. 8*c* is that it represents a multiple form of karyokinesis. The difficulty of determining the nature of such conditions will be readily understood, when it is remembered that the safranophilous bodies are usually not 2μ in diameter, and that, consequently, its metamorphic elements must be very small.

If the determination of the division of the nucleus is difficult, much more so is that of the full history of the spores. They are so small at first that, apart from the mother organism, they cannot be distinguished from other cellular contents, such as the swallowed portions of the debris of neighboring cells and the spore stages of other parasites. It is only in a few cases that circumstances favor the determination of some of the forms after they have escaped. In Fig. 1, for example, is shown a cavity in the interior of a cell, evidently once occupied by the parasite in question, and in the neighborhood of the cavity is a number of bodies like plasmosomata, of similar, or nearly similar size. These are evidently the spores derived from the organism which occupied the cavity. In a few instances, with the best conditions for observation, forms, like those shown in Fig. 9*a*, are seen. Here the structures are comma-shaped, and their resemblance to other forms in the same Figure, to that of Fig. 10*a* and to those in Fig. 2, is such as to suggest a developmental relationship. The probability, however, that very young forms of Sporozoan parasites are similar to those represented in Fig. 9*a*, is sufficient to invalidate any conclusion that might be drawn from this resemblance.*

There is more certainty in regard to the larger comma-shaped forms, such as are shown in Figs. 2 and 10*a*. These are intensely safranophilous bodies, and measure from 3 to 6μ. Their outlines are sometimes distinct, sometimes not, this depending on the way in which the organism is disposed in the field of the microscope. If the tail should happen to be above or below the head of the comma the organism may be recognised with difficulty. The connection between these and the spherulating forms can be seen by glancing at Fig. 10 *a–h*. In further development the head of the comma enlarges, the safranophilous substance collects into a small round mass, leaving the protoplasm which contained it more or less coarsely reticulated or finely granular, and with feeble staining capacity. The tail still retains its safranophilous character and remains distinct for several stages. The space between it and the head tends to increase when its point becomes applied to the head (Fig. 10*c*). At the same time it becomes somewhat elongated (*d*), and the safranophilous substance in it condenses into a thin band bounding the convex side of the crescentic cavity. The head also undergoes further changes (*e*). The protoplasm becomes collected at its periphery as a rim to which the small round safranophilous mass, the nucleus, is attached by delicate protoplasmic strands. In the next stage protoplasmic strands may stretch across the crescentic cavity, to the remains of the tail or the point of the

*Compare with Steinhaus' Figures of the intracellular parasites in the pancreas of the Salamander, Ziegler's Beiträge Zur Path. Anat., Bd. VII., Taf. XI.

tail may fuse with the head; in the latter case the crescentic cavity persists (*l*). The safranophilous substance gradually disappears from the thin band representing the remains of the tail, till finally its staining capacity is scarcely marked in some of the forms, although its density is noticeable. This sketch of the organism developed out of the comma-shaped body explains thus the occurrence of a denser, frequently more deeply staining zone at one side, the presence of a crescentic cavity, or of a cavity next the zone, and the frequently excentric position of the nucleus in the adult organism (Figs. 3 and 4). In individual cases, in which these peculiarities are apparently wanting, it may be that they cannot be observed, because the organisms are not favorably placed in the microscopic field.

We can, I think, now account for many of the forms shown in Fig. 9, especially those in which a deeply stained crescent occurs with a sphere in its cavity—they are merely comma-shaped parasites in the process of transformation into that stage in which sporulation takes place. In the same way we may explain some of the forms illustrated by Lukjanow,* especially his Figs. 14, 15, 16, 61*a* and *b*, 66, 72, 74 and 75, and probably also Figs. 7, 11, 13, 68, 69, 77 and 94. His Fig. 48 would seem to indicate that he saw the sporulating phase of the same organism. All his studies were made on the gastric mucosa of the salamander. I have found in the gastric mucosa of *Diemyctulus* very few abnormal structures of this character. If they are parasitic, their comparative absence from the stomach may be attributed to the digestive and resistent action of the gastric mucosa, and it is probable that the irregularity and atypical character of many of the structures drawn by Lukjanow may be due to the physiological action, during life, of the glandular elements in which they occurred.

It is interesting to note the structure of the cytoplasm around the full-sized organisms (Figs. 3–7). It is constituted of very fine rodlets, each with a thick end directed towards the organism and passing in a radiating manner peripherally into a zone of what appears to be finely granular protoplasm, but which is, probably, a portion of the cytoplasmic reticulum condensed. The border of thickened points in many cases closely resembles a membrane. It depends, apparently, on the vitality of the cell whether the radiating arrangement of the cytoplasm occurs or not. It may be absent, as in Fig. 2, when the cell shows signs of degeneration. It is difficult to understand the function of this mechanism, but we may suppose it to act as a filtering apparatus.

Op. cit.

II. ON CHROMATOPHAGOUS AND OTHER INTRACELLULAR PARASITES IN THE INTESTINE OF NECTURUS LATERALIS.

In the intestinal epithelium of *Necturus* are often found forms which, from their peculiarities, must be regarded as parasitic. When I observed them first, I considered them to belong in a general way to that class of intracellular structures which Lukjanow* has described as occurring in the gastric mucosa of the salamander, and of which there are not a few examples in the intestine of *Necturus*. They are well shown in preparations made from recently captured animals, and their characters are preserved well in the tissues fixed with Flemming's Fluid, or corrosive sublimate, and stained with alum cochineal, or haematoxylin or eosin.

The chromatophagous forms have usually an irregular outline and the protoplasm extended in one or more long pseudopodial processes, which taper often to fine threads. In some cases the whole organism is thread-like (Fig. 15 *p*). They are easily distinguishable in alum-cochineal preparations in the unstained, epithelial cytoplasm, in which they may be found, and by their stain being in every respect similar to, and as deep as, that of the chromatin bodies of the epithelial nuclei. With high-powered objectives the stain is seen confined to the fine granules which densely crowd the cytoplasm of these organisms. There is sometimes a quantity of unstained protoplasm at the thicker end (Fig. 14 *p*), or a more or less curiously shaped mass may lie in its neighborhood (Fig. 16 *pr*). Sometimes the bodies are found in the interior of nuclei, but, as a rule, they are not easily recognizable in this position, unless they show amœboid outlines or are fixed in the act of migrating from the nucleus. One is shown in the latter condition (Fig. 15 *p*). The nucleus is in this case partially deprived of its chromatin by the parasite, which owes its staining capacity to the chromatin it absorbs or invaginates.

An explanation of the relations of such structures as are shown in Fig. 13 (*p*) can be at best only problematical. Here two parasites, each in a separate cavity in the cytoplasm, have their prolongations hooked around one another. This is only one of several instances observed of such a condition, but the preparation drawn shows the process most distinctly. It may be a case of conjugation.

There are a number of forms which are either wholly unstained by the coloring reagent, or which possess one or more stained spherules or granules (Fig. 13 *p*). These may, in some cases at least, represent young stages of the chromatophagous forms.

*L. c.

In Fig. 12 is shown a cell from the base of the epithelial layer, which has certain peculiarities worthy of note. In one of its two nuclei is a cavity containing an eosinophilous, dumb-bell-shaped structure. The chromatin of this nucleus is very much condensed, but a portion of it is extended into the cavity in the form of doubly-beaded rodlets. The structure here reminds one strongly of that of the cytoplasm about the parasites in the intestine of *Diemyctylus* as described above, and I am inclined, therefore, to regard the dumb-bell structure as a parasite. The elements in the neighborhood of the second nucleus may be parasitic also. Such a case as this illustrates fairly well upon what slender grounds one has to judge of the parasitic or non-parasitic nature of some intracellular bodies.

III. On Certain Structures in the Pancreatic Cells of Amphibia.

In the pancreatic cells of Amphibia are structures which, since their discovery by Nussbaum,* in 1882, have excited attention amongst a number of cytologists, on account of their supposed participation in the processes of secretion. From the fact that they presented resemblances in position and form to structures described by v. la Valette St. George and Bütschli, as occurring in the testicular cells of some invertebrates, Nussbaum gave them for temporary use the name nebenkerne. It will be seen from the description given below that these elements are not normal portions of the gland cell at all, and, therefore, do not merit the title, which has, since Nussbaum's paper was published, maintained its place in nearly all the publications on the subject. I do not intend to discard the term, however, because the full history of the structures have not been worked out, and they may really belong to a stage of a Sporozoan parasite, whose adult form may already be described and named. In that case the continued use of the term nebenkern applied to these elements is preferable to the coining of a new word for temporary service probably, and I will, therefore, not offer any further excuse for adopting it in this work.

If the elements in question were normal, it might be advisable to give them an English name equivalent to the word nebenkern, in which case the words "paranucleus," or "accessory nucleus" might suffice. The word *cytozoon*, on the other hand, is precluded, since it has been adopted by Gaule and his pupils to denote, according to their views, the elements in certain stages of cell metamorphosis or cell rejuvenescence.

According to Nussbaum's description, the nebenkerne are placed in the

* Über den Bau und die Thätigkeit der Drüsen. Arch. für Mikr. Anat.. Bd. XXI., p. 296.

protoplasmic portion of the gland cell, between the nucleus and the membrana propria; they are oval in outline, and either solid or more or less spirally twisted. There may be one or more in each cell, and when one only is present it is usually larger than the several taken together, which may happen to be in another cell. On the fourth to the fifth day after feeding the animal (salamander), they are present in every gland cell, while they may be found with difficulty, or not at all, in animals recently fed, and they are rare in animals which have fasted for a long time.

Nussbaum also found solid nebenkerne in the œsophageal glands of the frog, and in the exhausted, unicellular glands in *Argulus*, and thread-like ones in the pancreas of *Triton*.

As to the nature of these bodies, Nussbaum came to no conclusion. Ogata*, on the other hand, put forward a view which connected them with processes of secretion and cell renewal. According to his account, they are the plasmosomata of the nucleus, which have wandered into the cell protoplasm. The small nebenkerne are homogeneous, spherical, or elliptical in outline, often elongated and do not stain with hæmatoxylin, but they readily imbibe eosin, which, consequently, obscures their presence amongst the similarly stained zymogen granules. In the larger nebenkerne the chromatin substance is present, consequently they are either colored homogeneously violet or have one or more corpuscles colored deep violet to pure blue. The large nebenkern can, on the one hand, in old and exhausted cells, develop into a new cell, which, situated immediately adjacent to the membrana propria, pushes the disintegrating nucleus and remains of the old cell towards the lumen, and increases its own cytoplasm, in which zymogen granules appear; on the other hand, it may, in ordinary cells, break up into zymogen granules. It depends on the general condition of the gland, whether the nebenkern breaks up into zymogen granules, or developes into a new cell. The production of zymogen is not, however, limited to the nebenkern, for the granules were seen in the process of formation in the nucleus.

Ogata found in the moderately large, as well as in the full-sized nebenkern, cavities and fissures which gave them various appearances. Sometimes the structures were seen to sit cap-like on the nucleus.

Ogata stimulated the pancreas either by pilocarpin or by electrical irritation of the medulla, and found the number of nebenkerne greatly increased. When two or more doses of pilocarpin were given at intervals of twenty-four hours, the resulting number of nebenkerne was smaller

*Die Veränderung der Pankreaszellen bei der Secretion. Arch. für Anat. and Phys., Phys-Abth., 1883, p. 405.

than when only one dose was given. He explains this on the ground that the first dose has greatly increased the number of nebenkerne, and thereby weakened the cells, which now respond to the second dose less readily.

Ogata also traced a relation between the disappearance of the nebenkerne and the appearance of new nuclei.

Platner's* first published views coincided to a certain extent with those of Ogata. His description substantially is this: The large round nucleolus of the pancreatic cell elongates, and moves towards the periphery of the nucleus, often pushing out its membrane. The long axis of the nucleolus corresponds to the radius of the nucleus. A portion of the nucleus becoming constricted off, this part contains the nucleolus and is separated from the main portion by the formation of a homogeneous, septal wall. The nucleolus and the separated portion of the nucleus constitute together the nebenkern, which, when the main portion of the nucleus regains its usual size, sits on it like a demilune. The nebenkern becomes homogeneous, separates from the nucleus and breaks up into granules which are probably zymogen. These observations were made on the pancreas of *Anguis fragilis*, and were corroborated in that of the frog.

Platner's second study† led to somewhat different results. He used for this purpose the pancreas of a number of Reptilian and Amphibian forms, but he obtained the most decided results from that of the salamander. In the latter the irregularly contoured nuclei of exhausted gland cells stain deeply with safranin, so that the nuclear framework becomes indistinct. Of the many or several prominences on each nucleus one only remains finally. Into this the chromatin, distributed throughout the nucleus, wanders, with the result that the prominence appears as a dark red bud on the remaining portion of the nucleus, which now gradually returns to the normal condition, namely, that in which the nucleus shows an unstainable caryoplasma (Kernsaft). These buds are variously shaped, large or small, round or irregular. The nuclear membrane in most of the cases still covers it. Often it has vanished and the contents, still colored deeply, lie as fibrillar or coiled elements, or as partially granulated material, in the protoplasm of the cell. The constriction between the nucleus and the bud deepens, till finally they separate, the bud now losing its uniformly staining capacity. At the same time the protoplasm of

*Über die Entstehung der Nebenkerne und seine Beziehung zur Kerntheilung. Arch. für Mikr. Anat. Bd., XXVI., p. 343.

†Beiträge zur Kentniss der Zelle und ihren Theilung. Arch. für Mikr. Anat. Bd. XXXIII. p. 180.

the cell increases, till it attains its normal maximum volume. The retrogressive metamorphosis of the nebenkern, as Platner now terms the separated bud, goes hand in hand with the vigorous formation of zymogen granules in the cell. The nebenkern stains less readily with hæmatoxylin, and its volume decreases gradually, till either only fibrillar remains of the same are visible among the zymogen granules, or it is indistinguishable.

It is seen that these observations raise the question how far "partial" chromatolysis, as Platner terms the formation and degeneration of the nebenkern, thus described, enters into the processes of secretion, but Platner leaves the matter undecided.

Platner accounts for the discrepancies in the two descriptions of the mode of formation of the nebenkern by stating that in the pancreas of Anura, which formed the basis of his earlier observations, the determination of the various points is difficult, because of the small size of the cells in which the nebenkern sits cap-like on the nucleus.

Steinhaus* solves the question of the nature of these bodies differently. He denies their normal occurrence in Amphibia. They were not present in the pancreas of six axolotls which he examined and they were also absent from the pancreas of frogs obtained from one locality, though present in those of another. Even in the pancreas of some salamanders they are absent. He states that they have no connection with the processes of secretion, as the formation of zymogen granules goes on as well in the cells deprived of these bodies, as in those possessing them. They lie unchanged and, so far as the formation of zymogen granules is concerned, inert in the cell protoplasm. He never saw any structures which proved either the origin of these bodies out of the constituents of the cell, or their conversion into zymogen granules, or their connection with cell renewal. Steinhaus studied the condition of the nucleus in all the phases of secretion, but could observe nothing which would be considered as nuclear budding, according to Platner's description.

Steinhaus gives no verbal description of the nebenkerne, but in his figures he represents them as varying in size and number in each cell and as thread or worm-like forms more or less coiled, some of the larger ones of which have one end of the thread thickened to resemble a head.

Steinhaus considers these bodies as parasites whose relationship to the Hæmatozoa is unmistakable, but so long as we know only this stage

*Ueber parasitäre Einschlüsse in den Pancreaszellen der Amphibien—Ziegler's Beiträge zur Path. Anat. und zur Allgem. Path., Bd. vii., p. 367.

in their life-history, it is impossible to say anything more definite than that everything at present determined points out their kinship to the Sporozoa

It is of interest here to note the occurrence of supposed nebenkerne in the pancreas of the dog.* Melissinos and Nicolaides found that these are intra- as well as extranuclear forms, sometimes of curious shape and composition. The intranuclear ones, the plasmosomata, may wander from the nucleus into the cell substance, where, as these observers are led to believe from the results of experiments with pilocarpin, they break up into zymogen granules. They deny the correctness of Platner's view, that the appearances, from which Ogata was led to believe that nuclear plasmosomata migrate into the cell, are artificially produced, and, in support of their position, they mention that in a quarter of an hour after the administration of pilocarpin the plasmosomata show all the stages of migration from the nucleus, the extranuclear forms are numerous and zymogen granules are present, while in half an hour after the administration, neither plasmosomata, extranuclear forms, nor zymogen granules are visible. The extranuclear forms, which arise by migration from the nucleus, of the plasmosomata, they call nebenkerne and these they distinguish from others, which are more or less complicated in their structure and composition and which lie in distinct cavities in the cell protoplasm. These latter they think are: (1) excretions of the cell protoplasm; (2) the remains of leucocytes; (3) chromatolysed nuclei.

METHODS OF STUDY.

I used several methods at the outset of this research but finally gave the preference to one mode of preparation which included either Flemming's Fluid or corrosive sublimate as the hardening reagent. This mode of preparation was as follows:

The animal (*Diemyctylus viridescens*, *Amblystoma punctatum*, *Plethodon glutinosus*) was decapitated, the abdominal cavity opened, the pancreas snipped away and immediately dropped into a saturated solution of corrosive sublimate, where it remained ten to fifteen minutes, or into a quantity of Flemming's Fluid, where it was left from one to twenty-four hours, according to the need. The operation of removal was usually done within twenty seconds, this interval including the decapitation process also. The object of this was to prevent any post-mortem

*Untersuchungen über einige intra- and extranucleare Gebilde im Pankreas der Säugethiere auf ihre Beziehung zu der Secretion. Von C. Melissinos. Mitgetheilt von R. Nicolaides. Arch. für Anat., und Phys., Phys. Abth., 1889, p. 317.

changes in the pancreatic cells and I believe that it was attained in every case. The piece of tissue was after removal from either of these fluids, washed for a few seconds in distilled water, then transferred to 70% alcohol for three hours, in the case of the corrosive sublimate preparation, and for twenty-four hours, when the Flemming's Fluid was used. When the latter was allowed to act longer than one hour, the alcohol was changed as often as it presented a trace of chromic acid coloration. The hardening was completed by a stay of twenty-four hours in 95 per cent alcohol. The organ was now transferred to the staining fluid, alum hæmatoxylin, (a few drops of a saturated solution of hæmatoxylin in absolute alcohol to a saturated solution of pure ammonia alum in distilled water: allowed to stand one month in summer sunlight before using, and kept from deterioration by crystals of thymol), for ten to fifteen hours. In order to prevent overstaining, I found it advisable to dilute the original hæmatoxylin solution with twice its volume of distilled water, in which dilution, after the time allowed, there is only a pure chromatin stain in the nuclei of the pancreatic cells and a faint shade of purplish blue in the nebenkerne. The objects are now washed in distilled water to remove the alum and the excess of the staining fluid, and are then put in a quantity of a 1 per cent solution of eosin in 30 per cent alcohol for from two to three hours. Washed in 95 per cent alcohol, till the latter was but faintly colored with the eosin after one hour's action, the object was placed in absolute alcohol for five minutes, then in pure chloroform for fifteen hours on the average, after which it was kept in a saturated solution of paraffin in chloroform at 35°C. for about eight hours, and finally placed for a like period in melted paraffin (melting point 52°C). The sections were made of a thickness not exceeding 5μ with the Thoma-Yung microtome and fixed by the ribbon method in series to the slide with a diluted Schällibaum's clove oil-collodion mixture, (clove oil 1 volume, collodion 3, equal parts of absolute alcohol and ether 3). I used, sometimes in the case of the corrosive sublimate preparations, the Gaule method of fastening the paraffin sections to slide, but, as the process of staining on the slide was not employed, except when the action of saffranin was required, it did not present any points of advantage over the other, which was the quicker. The paraffin was removed with benzole and the sections mounted in benzole balsam.

The staining of the object as a whole with hæmatoxylin and eosin has the advantages of giving a regular and uniform depth of reaction in the various sections and the different parts of each, and of preventing the loss of important elements entailed by the process of staining on the slide. I found that a little practice enabled one to judge of the length

of time necessary to give the tissue its proper depth of stain, and I determined that a stay of eight or ten hours longer than usual in the diluted hematoxylin solution, did not seem to increase the depth of the stain, or to make it more diffuse. Probably the explanation of this is that the equilibrium between the coloring matter in the diluted solution and that deposited in the tissue is reached when the chromatin is saturated. This, of course, is merely an application of the principle, that length of time and degree of concentration are elements in the right employment of staining methods and that these are, roughly speaking, in inverse proportion to one another.

In order to determine if the nebenkerne contribute in any way to the elaboration of the secreted elements of the pancreas I resorted to the use of pilocarpin. I had a large number of *Diemyctyli* at my disposal, and on these I studied the action of the drug, so far as the nebenkerne are concerned. Batches of ten, twenty and thirty were taken, and into the abdominal cavities of each of these less than 2 mgrm. of pilocarpin was injected. Three of these were, at certain periods after the injection, decapitated, the pancreas of each removed, hardened with corrosive sublimate, and treated as described above. These periods were usually: 1, 2, 3, 4, 5, 7, 9, 12, 17, 22, 36, 44, 52 and 60 hours, and these were chosen in some cases for convenience. I took three at each period, because, if I depended on one, misleading results might be obtained. It was found that the averages of the results obtained from each three agreed with each other in presenting an unbroken outline of the history of the nebenkerne.

I treated very young forms of *Amblystoma punctatum* also with pilocarpin, the method of employment of the latter in this case being to dissolve twenty to fifty milligrams in about half a litre of water and placing the animals therein for a period of five to twelve hours. As they measured between thirty and thirty-five millimetres in length, it is obvious that an intra-abdominal injection of a solution of the drug was out of the question.

The specimens of *Necturus* kept in the laboratory aquarium were not used for this investigation, since, owing to their not having been fed for a long time, the pancreas presented a more or less atrophied condition. It was found impossible to stimulate the gland in these to activity, or even to make it secrete at all.

There is a great advantage to be obtained from the concurrent use of the two hardening reagents, corrosive sublimate and Flemming's Fluid. The former fixes thoroughly and quickly the zymogen granules as well as the cellular and nuclear structures in the pancreas, while with Flem-

ming's Fluid, though the cell structure and nucleus are well preserved, the zymogen is dissolved out of all the cells except those at the immediate periphery of the organ. This removal of the zymogen is due to the acetic acid in the fluid, which penetrates where another constituent of the same mixture, osmic acid, is unable to diffuse. The action of acetic acid in this reagent enables us to distinguish between zymogen and other granules which have the same staining capacity with eosin. The osmic acid, furthermore, gives a dark tinge to the nebenkerne and unusual bodies in those cells near the periphery and thus brings them out in clear contrast to the other cytoplasmic structures.

OBSERVATIONS.

In sections made from the pancreas of *Diemyctylus*, which has been hardened with Flemming's Fluid and stained with hæmatoxylin and eosin, one observes in addition to the nucleus and cell protoplasm and, sometimes, zymogen granules, other structures which can be ranged in two groups at least. One of these groups comprise forms whose fundamental structure elements are thick or thin fibrillæ, either in sheaf shape, or wound in a ball fashion (Fig. 1). Sometimes the fibrillæ may be so thick as to merit the designation threads (Fig. 8). These forms are usually but not always, placed between the nucleus and the membrana propria, and they frequently sit, cap-like, on the nucleus, or the latter may be indented by them. In the second group, which are, at the outset, unlike the first, in that they are placed in cavities of the cell, are structures which present a varied form and composition. They are sometimes eosinophilous, sometimes chromophilous, and at times they present both characters, They are numerous in the pancreas of a freshly captured animal, but are not so much so as the members of the first group.

The members of these two groups of intracellular elements have been confused by other observers, and Ogata describes them as derived from the plasmosomata migrated from the nucleus, while Steinhaus appears to believe they are all parasites. In order to show that the views of these observers are hasty generalizations from a limited number of results, I propose to go fully into the description of the structure origin, mode of production, and history of each group. As plasmosomata, migrated, or extruded from the nucleus, are sometimes present, and as they have a different history, they merit special attention as a third group. These three groups may then stand in the order of description as follows:

1. Parasites.
2. The remains of broken down cells and nuclei swallowed by healthy adjoining cells.

3. Plasmosomata, migrated, or extruded from the nucleus into the cell protoplasm.

1. Parasites.

These are, as already said, usually, but not always, placed between the membrana propria and the nucleus of the cell. They vary in size, measuring in their extreme limits 1μ and 9μ, and their shape, usually oval, may also be oblong, spherical, elongated, club-like, or crescentic in section. They are not very sharply separated from the protoplasm of the cell and if the latter is dense, their outlines are distinguished with difficulty. Their structure varies also, but there are certain features in this respect which are tolerably constant for the great majority of these forms. These are the central cavity and the fibrillated appearance, the fibrillæ, as a rule, appearing as if wound around the central cavity. The central cavity may contain from one to several zymogen-like granules. The fibrillæ do not appear as if wound tightly, but are more or less tortuous in their course and the outermost ones may appear ragged, or project loosely into the surrounding protoplasm. This fibrillated arrangement is best seen in Flemming's Fluid preparations from freshly captured *Diemyctyli*, and, especially, in those on which the reagent has been allowed to act for twenty-four hours. The osmic acid and the hæmatoxylin in such give these bodies a dark brown stain, which deeply contrasts with the lightly or non-stained, surrounding protoplasm. In corrosive sublimate preparations, on the other hand, the fibrillation usually does not appear so distinct except under high powers when it readily becomes manifest, and hrimatoxylin gives it a faint reddish violet stain. Zymogen granules are entangled in the peripheral fibrillæ, often so abundantly, that they obscure the presence of the organism in question.

This stage is the most common, but in order to understand its nature, it will be necessary to consider the characters of the other forms found even in the same sections. These present more the appearance of plasmodia, are usually much smaller, and they take a deeper and more uniform stain with eosin. In the protoplasm of these, one can, at times, see concentric laminated slits, which are apparently an indication of a tendency to form fibrillæ, but which may also indicate that these plasmodia-like masses are derived by the fusion of the protoplasm of a coiled thread. Such coiled threads are rarely seen in ordinary preparations, but very frequently in sections from the pancreas of some *Diemyctyli*, which have fasted for about two months (Fig. 8). These coils have been, now and again, found to be dense in sections from the pancreas removed fifty to sixty hours from the animal after the injection of pilo-

carpin. When these bodies are very small, the number of turns in the coil is not more than two or three, whereas in the largest forms the number of turns cannot usually be made out.

All the forms, then, are either plasmodia-like masses, or are composed of fibrillæ or threads. Whether the plasmodia are elements of a separate stage in the metamorphosis of the bodies, or whether they are merely formed by the fusion of the protoplasm of the threads, cannot be decided definitely. It can certainly be determined that the fibrillated stage is one of degeneration, for one can find the fibrillated forms in all conditions up to disappearance. Figs. 1, 2, 3, 4, and *6nb* show this. The first step in this consists in a more or less parallel straightening of the fibrillæ and a consequent flattening of the whole mass, then the cell protoplasm pushes it towards the periphery where it lies, usually, directly under the cell membrane (Figs. 2, 3, and *4nb*). Here the fibrillæ disintegrate one by one, till finally, owing to their fineness and small number, they can not be distinguished from the cell protoplasm. Platner has described the occurrence of such fibrillated remains in the cell protoplasm, and he considers them derived from the nebenkerne.

I am inclined to believe that the coiled thread is the intact form of the parasite, and that the plasmodium-like mass may be either an earlier or a subsequent stage in the life history of the parasite. In the case of the latter form, the fact, that it is usually smaller than those in which the fibrous or fibrillated structure is manifest, tends to show that it is a younger stage, but not conclusively, since even small fibrillated masses occur sometimes.

I have withheld the proofs that these forms are parasitic till now. Of course each fact adduced is not of itself sufficient to prove the correctness of my view, but all taken together are conclusive in this respect. These facts may be summarized in the following items :—

They are not present in the pancreas of the great majority of young forms of *Amblystoma punctatum*. I sectioned the whole of the pancreas of seven of these and found these bodies in only two of them. Of these two, one contained only eleven of the structures, while the rest possessed hundreds, and in both these cases, as well as in the other five, the cells exhibited all stages in secretion. I treated five other larvæ with pilocarpin, and examined the pancreas at intervals of four, seven, eleven, thirteen, and twenty-two hours after, without finding a single specimen of this nebenkern. The larva, in which the greatest number of such were found, measured in total length a little over thirty millimetres, while the others were of the same length or some what longer, and we may conclude, therefore, that the occurrence of these bodies does not depend on the stage of

development although it may depend on the change in the food, or habitat, which the increased development entails.

2. They are present in all the cells of the actively secreting pancreas of *Diemyctylus*, as well as in that of an animal fasting for two months or more. When two or more are present in a cell, they are, usually, but not always, small. I have found them present in the cells apparently without diminution in number at every indicated interval, after the injection of pilocarpin. In corrosive sublimate preparations of the gland cells distended with zymogen granules, these bodies are, in many cases, not seen. If one relied wholly on corrosive sublimate as a hardening reagent, one might conclude that this is a stage in which the nebenkerne are absent, having been used up in the formation of zymogen, and such a conclusion has been advanced by Ogata. That the bodies are not absent, but merely obscured by the granules, is shown in preparations made with Flemming's Fluid from a pancreas in the same condition. This reagent dissolves out the zymogen in the centrally placed tubules, and, if allowed to act for twenty-four hours, blackens the structures in question, thereby showing them to be as numerous in this phase of cellular activity as in any other. I have, however, found that they, as a rule, stain somewhat more readily with eosin at certain intervals after injections of pilocarpin, and this condition is concurrent with the filling up of the exhausted cell with zymogen, and with a subsequent exhaustion of the same. The deeper stain during the formation of zymogen is due to absorption of the latter diffused from the nucleus, its seat of formation, while, in the other case, the cells, having their energy exhausted, cannot destroy or disintegrate the organisms, which absorb the cell juices and thereby attain a greater readiness for eosin. I think this latter condition is in some way connected with the vitality of the animal, for it is less apt to appear in vigorous animals, and I found it best exemplified in sluggish ones, while in some cases, again, it appeared in forty-five to fifty-five hours after the administration of one dose of pilocarpin.

3. They are not derived from the nucleus by constriction and partial chromatolysis, as Platner describes, although other structures described farther on, with which these have been confused, may be so derived. I have examined series of sections made from the pancreas of over seventy *Diemyctyli*, exhibiting all the phases of glandular activity and yet I have never in a single instance seen the bodies in question, in any way, derived from the nucleus, nor are they plasmosomata which have migrated from the nucleus and have undergone a certain amount of extranuclear development, a thesis which Ogata adopts and defends. I have found extranuclear plasmosomata, and, as will be seen from the description

further on, traced their history, which is totally unlike that of the structures in question. Given, then, that they are derived neither from the cell protoplasm nor from the nucleus, the only remaining conclusion possible is that they come from without—in other words, they are parasitic.

4. The parasitic nature of these bodies is best shown by their form in the two young *Amblystomata* referred to above. Fig. 10 *a*, *b*, *c*, *e*, *f* (*nb*), represent the commoner types of these and a resemblance to a "wurmchen" type is readily seen in these.

5. The fibrillation and gradual disappearance of these bodies occur without any participation whatever in the processes of cell activity and secretion. There can be no doubt about the correctness of this, and moreover, Platner's description practically admits it, although he thinks that the desintegration of these bodies furnishes material for an increase in the amount of the cell protoplasm and, possibly, of its zymogen. It is not to be denied that the desintegration and possible assimilation of these bodies increase the cell protoplasm and may, therefore, very indirectly assist in the formation of zymogen.

The statements made by Steinhaus that these structures are not derived from the cell or nucleus, that they have no functional relation to secretion, nor have anything to do with cell renewal, I can, therefore, fully confirm. His observation that they are inconstant even in the same species, agrees with mine as to the young *Amblystomata*. His figures, however, of these bodies resemble but few of mine, and show the "würmchen" form to be more common than I have been permitted to see in my preparations. If Platner's statement is correct, that the fibrillar remains of these bodies can be observed in the pancreas of the salamander, it is evident that Steinhaus has overlooked the full history of the structures. Steinhaus is also in error in concluding that the parasites alone are the nebenkerne of Ogata or Platner, for bodies have evidently been included in this class by the two observers, which are not parasitic at all.

What are these parasites? Steinhaus believes that they are similar to, not to say identical with, those described under the names Hæmatozoa and Cytozoa. There are several facts which speak for the correctness of this view. The forms of some of them correspond with that found in the blood cells of the frog, the "würmchen" of Gaule and known as *Drepanidium ranarum* of Lankester. The latter is also to be found in the blood of *Diemyctylus*. Kruse* states, however, that it is not present in the blood of the tadpole and this fact is to be taken in connection with the absence,

* Virchow's Arch., Bd. 120, p. 553.

generally, of the pancreatic parasites in young *Amblystomata*, if an explanation is desired of the latter phenomenon. Furthermore, the degeneration and disintegration of the pancreatic parasites and the complete absence of the reproductive processes show that some other tissue is the breeding ground of the parasite, and their presence in every pancreatic cell points to the blood as their source.

The destruction of such large numbers of the parasites in the pancreatic cells seems to indicate that the pancreas of Amphibia is a protective as well as a secretive organ, and that it plays this part specially, since the parasites have not been found in any other organ after the most careful search.

2. Karyolytic and Cytolytic Products

These elements are few in some *Diemyctyli*, abundant in others, the latter especially in freshly captured animals. They are found only in groups of the cells at certain spots in the sections and they present characters which definitely distinguish them from the elements described in the foregoing section. Probably the best representation of these forms is given by a glance at Figs. 6 *chm*, 4 *rchc*, *pmeg*, 5 *pm*.

Their form is usually spherical or approximately so, and their size, as well as their structure, varies. They often consist of chromatin and eosinophilous substance, or simply of protoplasm which has a special affinity for staining reagents. Less commonly, they may contain eosinophilous granules like the zymogen granules, or these may be present with the chromatin masses. Apart from the occurrence of eosinophilous granules and the slightly stained protoplasm, the structure of these bodies is mostly varied by the quantity of chromatin present and the form which it takes. Sometimes the whole of the structure seems composed of chromatin (Figs. 3 and 6 *chm*), but more frequently the latter forms a small oddly shaped mass irregularly placed in the structure. One may see rings, rods, crescents, hooks, and spirals formed of this substance and variously disposed in the protoplasmic mass carrying them. These bodies usually lie in the cavities in the protoplasm of the containing cell, a peculiarity which readily brings them to view when their affinity for staining reagents is very slight. These elements are sharply distinguished from the parasitic bodies in that they never fibrillate and they, moreover, have a different fate. The latter can only be studied in the pancreas of freshly caught animals, and in those in which the various phases of the resting cells are being developed. In the active gland they may be numerous but as the resting phase of the gland cell is step by step being established they are found to become correspondingly smaller, the staining

with hæmatoxylin less vivid, while the larger bodies disintegrate and the fragments become scattered through the cell. The disappearance of these elements, the concurrent increase in the cell protoplasm and the appearance of zymogen granules are not matters of physiological relation. The removal, or rather the disappearance of chromatin, is on the other hand in some way connected with the abundance of chromatin in the greatly enlarged nucleus of the containing cell (Figs. 3, 4, 5, and 6). The nucleus may be somewhat distorted in its shape, and this is without doubt due to the abundance of the chromatin which it has absorbed from the elements in the cell. The processes of disintegration and absorption go on till finally in the resting gland cell a few protoplasmic masses, scarcely larger than zymogen granules, may remain.

The origin of these bodies is to be sought for in the broken down gland cell. Indeed one can see them so derived in the sections. In Figs. 3 and 5 are some of the remains (*cm* and *rohe*) of such disintegrated cells lying in the intercellular spaces, while the surrounding cells contain masses, which, from their position, are evidently swallowed portions of the same. The farther a cell is removed from these intercellular masses the freer it is from the intracellular elements in question and at a distance not greater than the diameter of a cell these may be absent altogether. In other words, wherever one finds the intracellular bodies numerous one can also in the same or in the next section find intercellular elements to indicate the place of origin of the former. It is quite possible that disintegrated leucocytes may give rise to the same, but I have seen no evidence of such, except, perhaps, in such forms as that represented in Fig. *6a*.

These bodies are also present more or less in the pancreas of all the young *Amblystomata* examined and they exhibit here also the same varying composition and structure.

These bodies do not participate in the processes of secretion. The presence of eosinophilous granules, like those constituting the zymogen, led Ogata to consider them as breaking up into zymogen and from the fact that the parasites may appear to contain zymogen granules more or less imbedded in them, he concluded that the latter are earlier phases in this formation of zymogen. These eosinophilous granules are not formed of zymogen, however, because in the more centrally placed cells in a section of the pancreas prepared with Flemming's Fluid, the zymogen granules are dissolved out by the acetic acid in this reagent, but the eosinophilous granules are not affected. This phenomenon has a bearing on the mode of secretion and I will, therefore, forego an explanation of it till I come to this subject farther on.

Nothing can probably demonstrate more effectually the non-secretory nature of these elements than the fact that they are present in the cubical cells lining the ducts and ductlets of the gland (Figs. 11 *b*, *chm* and 12 *rchc*). Nor are these bodies confined to the pancreas, for I have found them in the ephithelial cells of the intestine, in the liver, the kidney and cutaneous epithelium of *Diemyctylus* and *Necturus*. They indicate, however, how little of a tissue is normally lost to itself and how it husbands its waste material. It is, of course, on first view, surprising that the pancreatic cells should exhibit amœboid properties, but it is less so when we remember that the hepatic cells, which in sections have a definite and apparently fixed form, manifest in the teased out scrapings from the cut surface of the fresh liver amœboid movements.

3. Migrated or Extruded Plasmosomata.

Platner denies that the nuclear plasmosomata migrate, and, at first, I was inclined to this view. It is easy to see in well hardened sections of the pancreas plasmosomata driven by the knife from the periphery of the nucleus into the cell, the nuclear membrane torn, and the cavity previously occupied by the plasmosoma empty. This occurs chiefly when the plasmosomata are large and placed next to the nuclear membrane. The apparent protrusion of the nuclear membrane, in some cases, is really due to a shrinking of the same at every part, except opposite the plasmosoma, which offers a resistance. I found, however, as the investigation proceeded that there were phenomena which could not be so explained. For example, in the pancreas of a young *Amblystoma*, about one-fourth of the nuclei showed plasmosomata which were fixed in the act of passing from the nucleus to the cell. I saw plasmosomata of dumb-bell form half outside and half within the nucleus and some were embedded in the cell protoplasm. I saw this condition, moreover, but less marked, in the pancreas of a specimen of *Diemyctylus* removed twenty hours after the injection of pilocarpin. Though the evidence was unmistakeable, I cannot but think that if the phenomena are constant or normal, they should be observed oftener. In any case the migration or extrusion has, from all that I see, no connection with the processes of secretion. If it is a case of extrusion, one might imagine it to occur readily in the pancreas of any specimen of *Diemyctylus*, unless one were to suppose that in certain stages of cell activity the nucleus is more contractile. My attempts to establish the correctness of such a supposition resulted unsuccessfully.

That the extrusion or migration is not a normal phenomenon appears to be borne out in the history of the extranuclear plasmosomata. They either disintegrate and form granules like that of zymogen in size and

staining reaction, or persist for a time in a cavity of the cell protoplasm and gradually loose their eosinophilous character. Forms of the latter are rare but they can be distinguished from the cytolysed products of other cells by the fact that they are more or less eosinophilous, and by the fact farther, that one only is to be found in a cell, while similar bodies, protoplasmic or otherwise, are absent from the adjoining cells. For the purposes of the diagnosis of course serial sections are necessary. But with these aids even, the process of determining whether a slightly eosinophilous, extranuclear mass is a plasmosoma derived from the nucleus is a difficult one. The disintegration into zymogen-like granules is easily distinguishable on account of the fact that the resulting granules are collected at one spot in the cell (not near the border) and from their resisting the action of acetic acid. It is possible, on the other hand, that a plasmosoma may neither disintegrate into zymogen-like granules, nor persist with the gradual loss of the eosinophilous character in the cell protoplasm. I observed in the pancreas removed from an animal one and a half hours after the injection of pilocarpin, the ductlets filled with zymogen in a granular condition and containing here and there a large plasmosoma-like mass. In this case no intra-cellular plasmosomata were observed, although zymogen was still present in the cells. I think this phenomenon indicates that the pancreatic cell can, under such a strong stimulus as pilocarpin furnishes, throw out of itself all material not part of its own mechanical structure, and that the extranuclear plasmosomata may, in some cases, be disposed of in this way.

That Ogata made the mistake he did in assuming that the extranuclear plasmosomata become converted into nebenkerne and the latter again into zymogen granules is very natural in view of what is described above. The passage of plasmosomata from the nucleus to the cell, the mingling of zymogen granules, either with the substance of the plasmodium-like mass or with the fibrillæ of the degenerated parasite and the occurrence of protoplasmic masses loaded with eosinophilous granules are demonstrable facts which Ogata seems to have observed, and he built up from these the theory outlined, a feat and a mistake which any cytologist, who had paid as careful attention to the subject as Ogata did, might have committed at that time. What was less excusable was the construction of a theory of cell rejuvenescence, for although chromatolysis was then unknown, or at least undescribed, and, therefore, the occurrence in pancreatic cells of protoplasmic masses possessing chromatin unexplained, yet the knowledge concerning the indirect process of cell division had then made a great advance and it was hardly necessary to postulate the existence of another process. All things considered, however, Ogata's

work has been of great service in calling special attention to structures, the further study of which may definitely establish a new function for the pancreas in cold-blooded animals, viz., a protective one against the Hæmatozoic parasites.

In connection with these remarks on Ogata's views, I may mention that I have frequently observed in some sections of the pancreas of *Diemyctylus* examples of karyokinesis and that in the cells in this condition there were neither nebenkerne, protoplasmic masses, nor plasmosomata. Steinhaus gives an illustration of a pancreatic cell exhibiting karyokinesis in which, apparently also, nebenkerne (parasitic) are present. I have also frequently observed cell and nuclear division in the pancreatic cells of the young *Amblystomata* and it was apparent that the nuclear division might go on with the cell more or less filled with zymogen granules.

4. Zymogenesis.

It has been known from the researches of Haidenhain and others that changes in the shape and staining power of the nucleus accompany the change from the resting to the active phase of the secreting cell. What the relations are which these changes bear to one another, were not divined, but it was generally supposed that they were the results of increased or decreased nutrition. The observations of Platner and Steinhaus embrace one aspect of these changes *i. e.*, the staining power of the nucleus, and it is to this that I propose to devote this section.

A summary of Platner's views as to the changes in the staining power of the nucleus of the pancreatic cell has been given above in the historical sketch of the literature on the pancreatic nebenkerne. Steinhaus'* observations, bearing more directly on the staining power, are of greater interest to us and may be abstracted as follows :

The exhausted gland cells are small, indistinctly contoured, and deficient in protoplasm and their arrangement in the form of alveoli is lost. Their nuclei which are angular and crenated are, when a double stain of hæmatoxylin and safranin is employed, colored red, and their nucleoli are safranophilous. When the active phase of the cells begins, the cytoplasm increases, the contour of the cell becomes distinct, the arrangement in alveoli with central lumen is attained, while the form of the cell becomes bluntly conical. At the same time the nucleus becomes oval and stains readily with hæmatoxylin. This dye stains one sort of nucleoli, the karyosomata, while the safranin colors the other and larger kind, the plasmo-

* *op. cit. p.* 371.

somata. At this point the formation of zymogen granules begins in the part of the cell next the lumen and it proceeds till the cell is filled with them. As to the origin and mode of production of the zymogen granules nothing is known. When secretion begins these granules disappear and the nucleus now tends to return to the condition found in the exhausted cell.

My own observations coincide with those of Steinhaus. I may emphasize here one or two points. The nucleus of the exhausted gland cell stains readily and deeply with safranin, that of the cell in which the formation of zymogen is going on vigorously, is colored deeply with hæmatoxylin, while its plasmosoma takes readily the safranin.

My explanation of this phenomenon is drawn from the results of my observations on the formation of yolk in the ovarian ova of *Necturus* and *Rana*, and a summary of these may therefore not be out of place here.

In the nuclei of the developing ova at a certain stage the chromatin is principally collected in the form of nucleoli at the periphery immediately under the nuclear membrane. These nucleoli are usually spherical and they may, though not usually, or very much, vary in size. All the chromatin of the nucleus is not so situated, for there are long threads which at certain points in the granular karyoplasma unite at angles with one another. At this stage yolk spherules are absent from the cell. If now sections of such an ovary are stained with the indigo-carmine stain of Shakespeare and Norris, the significance of the peripheral nucleoli is determined. Such sections show here and there an ovum in which the peripheral nucleoli are stained deep blue, while the remainder of the nucleus and cell is stained red. In other ova, again, the peripheral nucleoli and the karyoplasma are stained blue, the cell red, while in others again the peripheral nucleoli are smaller, the whole ovum, with its yolk spherules which now begin to be formed, is stained blue, or blue green.

The origin of the substance which stains indigo-blue in this process is certainly derived from the peripheral nucleoli, for it is possible to meet with an ovum once in a while in which a portion of the karyoplasma in the immediate neighborhood of and around each nucleolus is, like the latter, stained indigo-blue, while the remainder of the karyoplasma is red. The peripheral nucleoli generate a substance, therefore, which diffuses gradually through the nucleus, then into the cell protoplasm, the point in time of the latter occurrence corresponding with the formation of the yolk spherules. The mode of origin is through a process of deposition from the nucleus of a substance allied to chromatin in the cytoplasm.

The diffusion of a substance produced from the nucleoli through the

nucleus and into the cell protoplasm, can also be determined by other staining reagents, *e.g.*, alum cochineal, but the different stages in this phenomena cannot be thereby so readily determined as with the other method.

I regard the yolk spherules as formed by the union of a derivative of the nuclear chromatin with a constituent of the cell protoplasm. This derivative of the nuclear chromatin is, possibly, the same as the hæmatogen which Bunge discovered in the fowl's egg united with an albumin. The formation of yolk spherules in the cell protoplasm is analogous to the the formation of zymogen granules in the pancreatic cells and both are accompanied by changes in the nucleus and an increase in the cell protoplasm. It is most natural to conclude that the processes underlying the formation both of the yolk spherules and of the zymogen granules are in a general way alike. We see many facts supporting this view. In the developing ovum there are phases in the elaboration of the chromatin and the formation of nucleoli (plasmosomata) comparable to the production of chromatin in the nucleus of the resting pancreatic cell, and to the apparent conversion of this chromatin into safranophilous substance which diffuses through the nucleus in the exhausted cell. We see a further parallel to the formation of yoke spherules in that as the nucleus loses its safranophilous substance the cell protoplasm acquires safranophilous granules. If we accept the parallel so far as correct, we may then assume that the chromatin of the nucleus of the pancreatic cell gives rise to a substance which we may call "prozymogen," sometimes dissolved in the nuclear substance, sometimes collected in masses (plasmosomata), and finally diffused into the cell protoplasm, uniting with a constituent of the latter as zymogen. This is, I think, the true explanation of the phenomena of secretion.

With the help of this theory we can explain why it is that in certain pancreatic cells the protoplasmic masses contain, as described above, eosinophilous granules of exactly the same size as those of zymogen, but unlike the latter in that they are not dissolved out by solutions containing acetic acid. The protoplasmic masses swallowed by a pancreatic cell, cannot be of the same composition as the cell protoplasm, and are not amenable to the laws which govern the nutrition of the cell as a whole. When the prozymogen diffuses from the nucleus to the cell it invades the protoplasmic masses enclosed, and it becomes united with a constituent of the latter, thereby forming a compound similar to zymogen in some respects: the capacity for forming spherules, the eosinophilous and safranophilous reaction, but differing from it, as already said, by being insoluble in solutions of acetic acid.

That the nucleus is the seat of formative energy is shown by a number of observations*, especially those which bear on the vegetable cell. In the latter the first stages, at least of starch formation, are carried out in the nucleus and this secretes a compound which is finally converted by the the cytoplasm into starch. Korschelt† has also determined that in the formation of the chitinous processes on the eggs of *Nepa* and *Ranatra* the chitin necessary for each process is elaborated in a cavity between and surrounded by two epithelial nuclei, and the only legitimate conclusion from such a circumstance is that the chitin is derived from a nuclear substance. I may also here refer to the fact that my own observations have definitely shown that the hæmaglobin of the red corpuscles in *Necturus* and *Amblystoma* is derived from the chromatin of the nucleus both of the fully formed as well as of the developing red cell, and that the hæmaglobin so formed diffuses through the nuclear membrane and becomes fixed in the cytoplasm. All these facts point definitely to the prominent part played by the nucleus and if everything in connection therewith is carefully studied, it will be admitted, I believe, that the interpretation which I have given of the changes occurring in the nuclei of the pancreatic cell during the various phases of glandular activity, is not a strained or a far-fetched one.

APPENDIX.

After the foregoing was written, a paper containing the observations of Eberth‡ on the pancreatic nebenkerne in salamander came into my hands. In this is advanced a new view of the relations of these bodies, or pseudo-nuclei, as Eberth prefers to call them. He states that they are developed out of the reticular fibrillæ of the cytoplasm, the latter at spots apparently becoming swollen, or thickened by fusion with their neighbors, and at the same time altered in composition, whereby their

* See a resumé of such researches in Strasburger: "Ueber Kern-und Zelltheilung im Pflanzenreiche nebst einem Anhang über Befruchtung," Jena, 1888, pp. 194-204.

† "Ueber einige interessante Vorgänge bei der Bildung der Insecteneier." Zeit. für wess. Zool. Bd. 45.

‡ Ueber Einschlüsse in Epithelzellen. Fortschritte der Medicin, Sept. 1., 1890.

capacity for absorbing staining reagents is increased. Later several of such bent fibrillæ approach one another and acquire the shape of a sickle, semicircle, or circle. The latter show all possible stages of transformation into the laminated bodies and spherules, which possess a very irregular fibrillation appearing to consist of loose threads, while they may at times resemble laminated colloid bodies. The pseudo-nuclei disappear during hunger, while becoming gradually paler and less easily stainable. As to the process and manner of disintegration Eberth could offer no explanation. He compares these bodies with structures described by Czermak as occurring in the ethmoid cartilage of the calf, and with those found by Solger in the cartilage cells of the shoulder-girdle of the pike. Eberth believes these structures to be normal, and in a sense, comparable to the nodules of the nuclear network.

Eberth states that the employment of corrosive sublimate as a hardening reagent and of paraffin for imbedding produces contraction and shrinkage in these objects, and that then one obtains the peculiar shapes which possess a certain resemblance to Cytozoa. He accordingly recommends Rabl's Fluid or Flemming's Fluid for hardening and celloidin for imbedding.

Now I have carefully gone over the whole of my preparations since last October, and have during this winter made a number of new preparations from *Diemyctyli* and young *Amblystomata*, using for this purpose each of the three hardening reagents mentioned, frequently on pieces of the pancreas from the small animal. I have found that Rabl's Fluid often gives the appearance of coarse, parallel fibrillation in the pancreatic cells, when neither Flemming's Fluid nor corrosive sublimate demonstrated the presence of a single nebenkern in the parts of the pancreas hardened with either of these reagents. Such a parallel arrangement of coarse fibrillæ is probably artificially produced. It appears also to cause a swelling of the cytoplasmic fibrillæ, whereby these are rendered more distinct, and I think that to this property is due the advantage obtained by the employment of Rabl's Fluid in demonstrating the elements of the achromatic spindles in dividing nuclei.

My later observations strongly confirm my view that the nebenkerne are parasitic elements. In eight *Amblystomata*, killed during January and February, there were nebenkerne in only one, and here very abundantly. There could be no doubt about the sharply outlined form, as Steinhaus has figured it, often homogeneous but as often fibrillated. I have seen quite distinctly the thickened portion of the organism which simulates a head. As the *Amblystomata* kept in the laboratory tank were

not regularly fed, I attribute the intact form possessed by many of the parasites to the lowered vitality of the host produced by want of food.

Eberth's views are directly opposed to mine. He considers the fibrillation of the structures in question not as an evidence of their degeneration, but as a stage in their formation. His observations, confined as they were to one form, cannot, I think, be held as conclusive by any one who has studied the changes in the pancreas of Amphibia as exhibited throughout the year. I cannot share Eberth's views as to the action of corrosive sublimate on the form of these bodies and that it does not produce a contraction or shrinkage, as he maintains, is shown by Figs. 1, 2, 9, and 10*d*, *nb*, which were drawn from preparations made with this reagent. I would call attention to Fig. 10*b*, *nb*, which shows a form not at all uncommon in the specimen of *Amblystoma* referred to in the last paragraph and which is very like some of the specimens of *Drepanidium* figured by Gaule.

I have, in this connection, made further observations on the elaboration of the pancreatic ferment. The results of these are confirmatory of the views already advanced by me and may be summarized as fo lows :—

1. In the gland cell filling up with zymogen granules, the latter are largest at the border of lumen of the gland tubule, while the smallest are found at that edge of the granular area nearest the nucleus. This serves to show that the granules are increased in volume by the deposition of a substance from the "protoplasmic" area of the gland cell.

2. While the eosinophilous substance disappears from the nucleus, the "protoplasmic" zone becomes eosinophilous at a time nearly coinciding with the commencement of the deposit of granules in the cell In other words, the eosinophilous (or safranophilous) substance diffuses out the nucleus to the protoplasmic zone of the cell, from which it is apparently removed to be fixed in some way in the zymogen granules.

3. In the gland cell after exhaustion and when a restoration of its active condition commences there is an absorption, apparently from without, of chromatin, or of a chromatin-like substance, by the protoplasmic zone, and it would seem that the nucleus increases its quantity of chromatin from this source.

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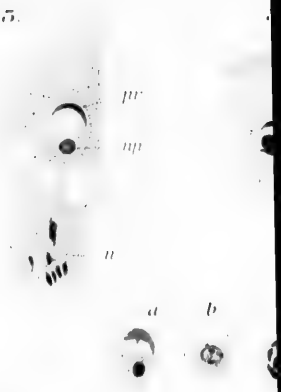

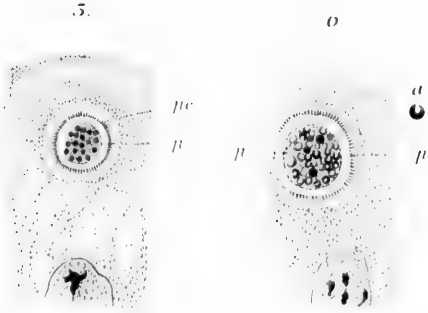

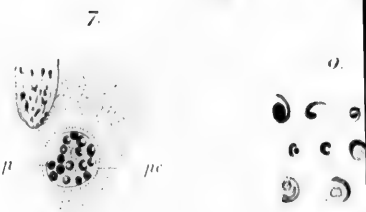

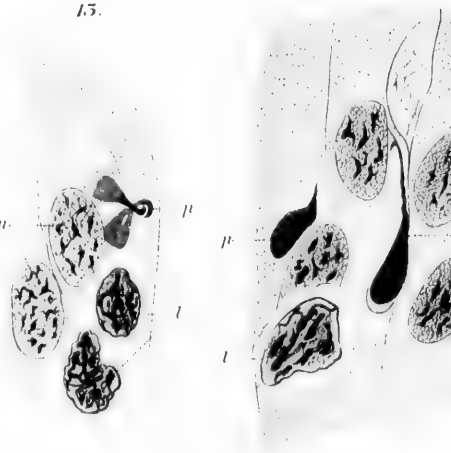

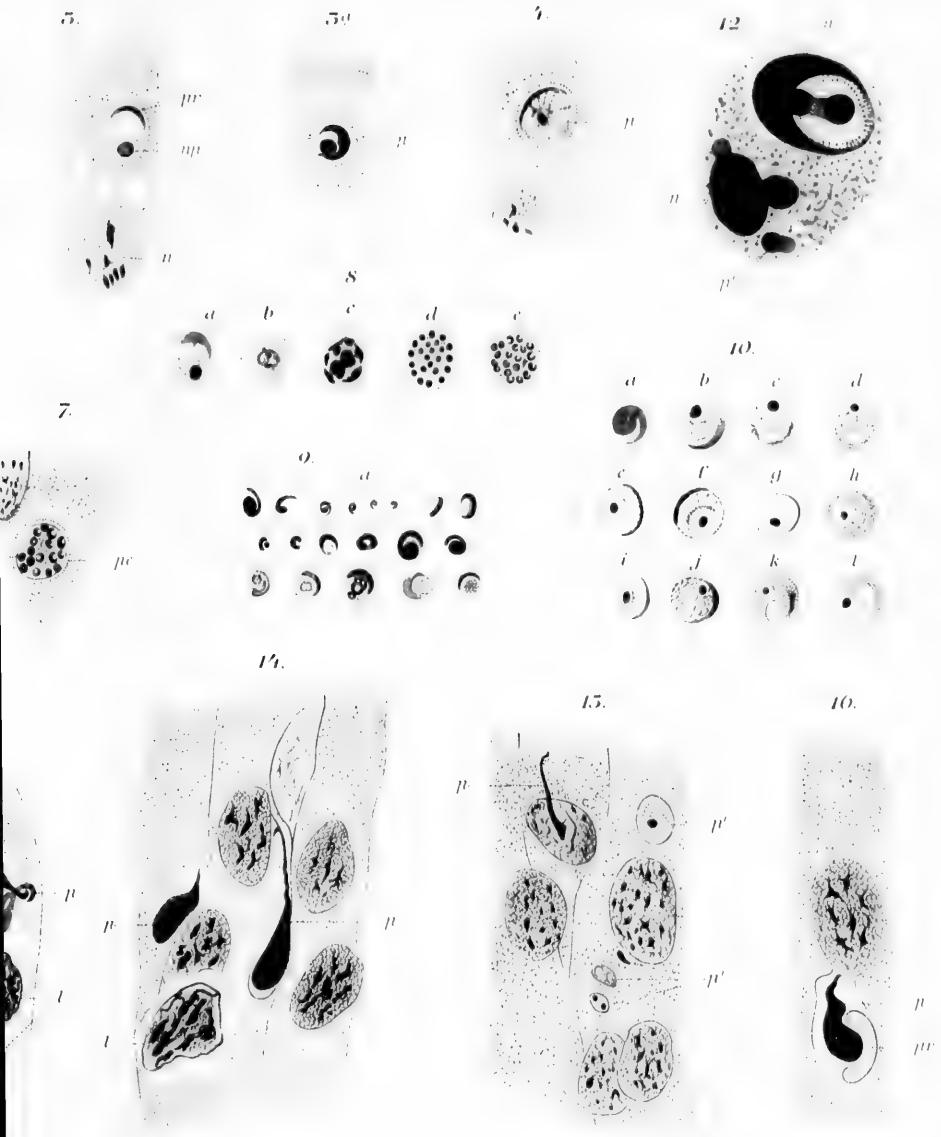
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Explanation of Plate I.

The illustrations are drawn with the Abbe camera lucida, combined with the 3mm. or the 2mm. apochromatic objectives (Zeiss), and compensation ocular 4 or 8.

Figs. 1-11 are from the intestine of *Diemyctylus viridescens*.

Fig. 1. Three epithelial cells in each of which there are unusual structures—*cp* represents the cavity in which, apparently, a parasitic element matured and whose spores are seen in the adjacent cytoplasm. In the central cell are probably both spores and invaginated, cytolysed material, while in the cell to the left there are structures which from their shape appear to be parasitic. ×720.

Fig. 2. Two epithelial cells in one of which the nucleus is degenerated. In both cells are seen structures exemplifying two stages in the development of the same parasite. In the degenerated cell the parasite, *p*, is matured, but in the cytoplasm of the other cell, they are, apparently, all comma-shaped. ×1000.

Fig. 3. A single epithelial cell containing a fairly typical specimen of the parasite, *n*, the cellular nucleus, *np*, the nucleus of the parasitic organism. There is present a cavity and a rim of thickened protoplasm, *pr*.

Fig. 3*a*. An epithelial cell in which the parasite, *p*, is in the stage of transition from the comma to the adult form. ×1000.

Fig. 4. In this the parasite, *p*, possesses a central mass of protoplasm in which is imbedded the nucleus and which sends processes toward the periphery. The remains of the tail of the comma are still recognisable in the denser portion of the periphery. ×1000.

Figs. 5, 6, and 7. The sporulation stages of the parasite with the trabecular arrangement of the cell protoplasm *pc*, well marked. The horseshoe form of the spore is clearly shown in 6*a*. ×1000. 6*a*. ×2250.

Fig. 8. Represents five stages in the development of the sporulation phase of the parasite. In *a* the thickened band of protoplasm at one side represents the remains of the tail of the comma stage; in *b* the two central rings probably represent a stage of mitosis which is further advanced in *c*; in *d* the spores are formed each in a cavity of the ptoor plasm and these are further developed in *e*. ×1000.

Fig. 9. Represents illustrations of comma forms met with in the epithelial cells. In *a* coiled form is shown. ×1000.

Fig. 10 *a-d.* are illustrations showing the way in which the comma is transformed into the adult parasite; *e-l* represent forms which show the various ways in which the nucleus, cavity and tail are disposed in the adult or developing form. ×1000.

Fig. 11*a*. Represents a section of an epithelial cell in a cavity of which are enigmatical structures, the larger one probably being parasitic, the others may be either parasitic or protoplasmic masses with chromatin spherules. ×1000.

Fig. 11*b*. In this cell are a number of structures all of which are evidently parasites. ×1000.

Fig. 12. A cell found in the epithelial layer of the intestine of *Necturus*, *n*, the nucleus, *p*, plasmosomata-like masses which may be parasitic, n^1, a nucleus in which the chromatin is principally massed at one side and continued into the cavity in the form of doubly beaded rodlets; a dumb-bell shaped body, deeply eosinophilous, is shown in the act of migration from the cavity. ×660.

Fig. 13. Epithelial cells of the intestine of *Necturus; n*, the epithelial nuclei; *l*, the nuclei of leucocytes; *p*, two intracellular parasites lying in cavities of the cell. ×660.

Fig. 14. Intestinal epithelial cells of *Necturus; p*, parasitic elements; *l*, the nucleus of a leucocyte. ×660.

Fig. 15. Intestinal cells of *Necturus; p*, parasites migrating from from nucleus; p^1, either invaginated, cytolysed material or stages in the development of the parasite. ×600.

Fig. 16. A single epithelial cell of the intestine of *Necturus*, showing a large cavity in its proximal part occupied by a parasite *p*, and protoplasmic remains, *pr*. ×600.

EXPLANATION OF PLATE II.

The outlines of all the figures were made with Abbe's camera lucida in combination with 2mm. apochromatic objective and compensation ocular, 4 or 8. In the case of Fig. 8 the drawing was made at the foot instead of at the level of the stage of the microscope, hence the difference in the magnification.

bc, blood corpuscle.

cm, cytolysed masses.

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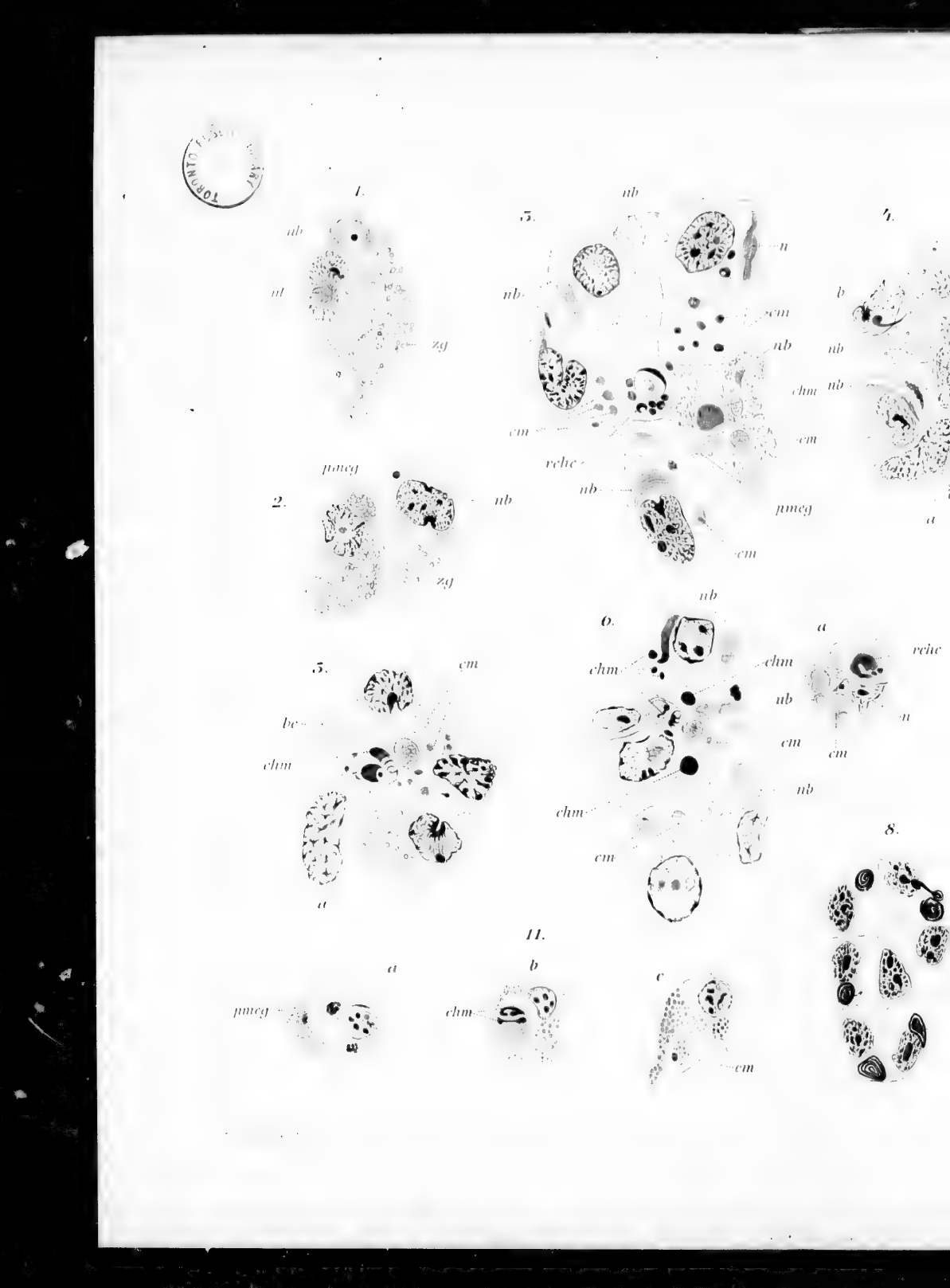
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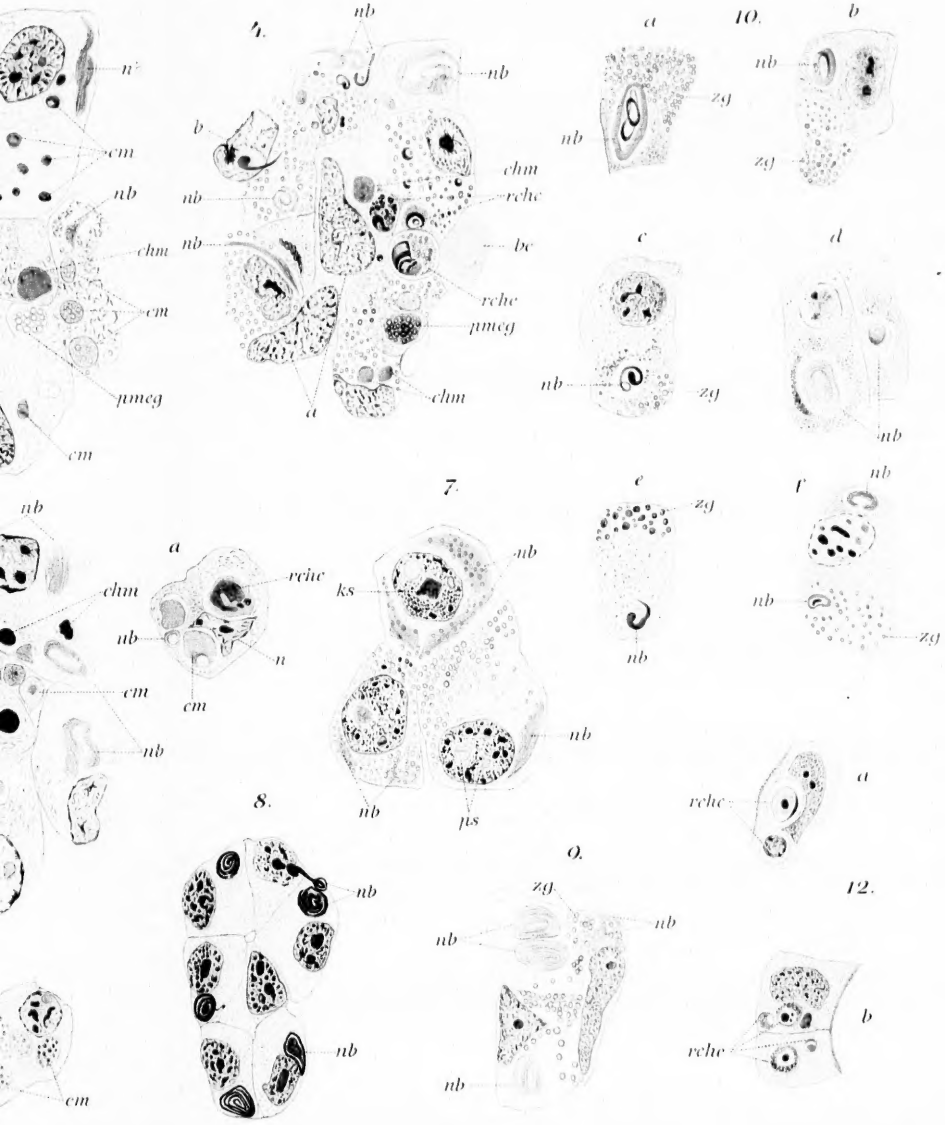
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chm, chromatin masses or bodies derived from chromatolysed nuclei.

pmeg, protoplasmic bodies loaded with eosinophilous granules like zymogen, but insoluble in acetic acid.

nb, nebenkern.

rchc, remains of chromatolysed nuclei and cells.

zg, zymogen granules.

Fig. 1 A resting pancreatic cell from *Diemyctylus; nl*, a large irregular plasmosoma; the chromatin is very abundant. Corrosive sublimate. Hæm., eosin. ×1000.

Fig. 2. Two resting pancreatic cells from the same preparation as the last. In the right hand cell the elasticity of the fibrils of the degenerated nebenkern has sprung out the cell wall. ×1000.

Fig. 3. From the active pancreas of *Diemyctylus*. Illustrates the invagination by normal cells of cytolysed material. The cavity in the centre occupied by the round mass, *rchc*, was probably the site of the cytolysed cell, and from this the cytolysed products have passed to the surrounding cells. The part represented occupied the centre of the section and the meshes of the cytoplasmic network were filled with zymogen granules which were dissolved out by the acid hardening reagents. It is to be noted that the nuclei here are large and rich in chromatin. Flemming's Fluid. Hæm., eosin. ×1000.

Fig. 4. Taken from near the margin of a similarly prepared section and therefore showing zymogen granules; *a*, enlarged nuclei; *b*, a nucleus with a sickle-shaped element, half within and half without the cell. ×1000.

Fig. 5. From the resting pancreas of *Diemyctylus*. The part drawn was from near the margin of the section. In the centre of the illustration is shown a cavity or intercellular space partially occupied by cytolysed material and the chromatin derived from it is found in the adjacent cell *(chm)*, whose nucleus is greatly enlarged. The other nuclei are somewhat irregular and rich in chromatin. Flemming's Fluid. Hæm., eosin, safranin. ×1000.

Fig. 6. From the central part of a section from the pancreas of a freshly captured specimen of *Diemyctylus*. Here also are shown free intercellular masses, and in the adjacent cells spherules of chromatin and cytoplasm; *a* represents a single cell from the same preparation. Flemmings Fluid. Hæm., eosin. ×1000.

Fig. 7. Three pancreatic cells from *Diemyctylus*. The formation of zymogen has advanced somewhat, the chromatin is abundant and the

karyosomata numerous and sometimes large *(ks)*. The plasmosomata of which there are two to each nucleus are usually large and irregular in shape. Corrosive sublimate. Hæm., eosin. ×1000.

Fig. 8. From the pancreas of a specimen of *Diemyctylus* deprived of food for five weeks. Corrosive sublimate. Hæm., eosin.

Fig. 9, From the pancreas of a specimen of *Diemyctylus* removed forty-five hours after an intra-abdominal injection of 0.4mgrm of pilocarpin. Corrosive sublimate. Hæm., eosin. ×1000.

Figs. 10 and 11. From the pancreas of specimens of *Amblystoma punctatum* (developing into adult condition). Fig. 10, *a-f*, drawn from the same pancreas. Corrosive sublimate. Hæm., eosin. ×1000.

Fig. 12. Cells lining the pancreatic ductlets of *Diemyctylus*, showing in their interior cytolysed and chromatolysed products *a* and *b*, from the pancreas 24 hours and one hour respectively after the intra-abdominal injection of pilocarpin. Corrosive sublimate. Hæm., eosin. ×1000